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CONNOLLY BOVE LODGE & HUTZ, LLP
P O BOX 2207
WILMINGTON, DE 19899

EXAMINER	
PANDE, SUCHIRA	
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1637	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/573,639	SCHWANEBERG, ULRICH	
	Examiner	Art Unit	
	SUCHIRA PANDE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 September 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-15* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-15* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claim Status

1. Amendment filed on September 18, 2008 is acknowledged. Applicant has amended claims 1-15 to correct the antecedent basis and to better comply with the U.S. practice. Claims 1-15 are currently active and will be examined in this action.

Response to Arguments

Re 103 rejection of claims 1, 4-7, 12-13 and 15 over Henikoff in view of Zaccolo et al.

2. Applicant's arguments filed September 18, 2008 have been fully considered but they are not persuasive. Applicant is arguing following points:

1) On page 7, Applicant is arguing about elements shown in Fig. 1a of specification. Applicant is reminded the instant claim language does not refer to Fig. 1a hence Applicant is arguing limitations that are not recited in instant claims.

2) Applicant argues that in Henikoff's method the (+) strand fragments never exist in a status of "single -stranded fragments". This is factually incorrect. (see page 2966 2nd par. where Henikoff teaches use of appropriate helper phage Mike or M13K07 to produce the single stranded sequence of both the + and - strands. So by using appropriate helper phage, single stranded (+) strand fragment will be made. Thus teaching that single stranded (+) strand fragment do exist in a status of "single -stranded fragments".

3) Applicant is arguing that steps (iii) and (iv) require the full length of master sequence must be synthesized. Examiner would like to point out that step (i) of claim recites creating a collection of single-stranded fragments of the (+)-strand of the master sequence wherein all members of the collection have the same 5'-terminus and have a deletion in the 3'-terminus such that the collection represents (+)-strands with a length of n-l, n-2, n-3,. .... nucleotides.

Thus at end of step 1, one will end up with single stranded collection of (+) strands with a length of n-l, n-2, n-3,. .... nucleotides. Thus only single stranded deletions of the master strand (+) now exist in this collection. So using these single stranded deletions of (+) strand as template the method can only generate the double stranded fragment of full length of the appropriate length n-1, n-2, n-3,---nucleotides. The method recited can not generate the full length of starting master sequence given the limitation recited in step (i) of claim 1. Only full length of corresponding (+) strand deletions that were generated at end of step (1) can be generated.

Applicant is arguing features depicted in Figure 4. Applicant is reminded that Figure 4 is not recited in instant claim hence, Applicant is arguing limitations that are not recited in instant claims.

4) Finally Applicant correctly points out that Henikoff's nested deletion method produces molecules that are shorter than the master molecule. This is exactly what is recited in step (i) of instant claim. When the starting template molecule is a single stranded deletion of master sequence then using these

deleted (+) strands as a template to make (-) strands will result in generation of only nested deletions.

Based on the currently recited claim limitation it is not possible to generate original full length molecules as the starting double stranded master sequence from which the fragments of step (1) were created. Therefore in Examiner's opinion, the cited art Henikoff is still applicable to the claims as currently recited. Hence the previously cited 103 rejections of claims 1, 4-7, 12-13 and 15 over Henikoff in view of Zaccolo et al. are being maintained.

Re 103 rejection of claims 2, 8-11 and 14 over Henikoff and Zaccolo et al. as applied to claim 1 above further in view of Krokan et al. and Short et al.

3. Since rejection of claim 1 over Henikoff and Zaccolo et al. is being maintained, hence the rejection of claims 2, 8-11 and 14 over Henikoff and Zaccolo et al. further in view of secondary references Krokan et al. and Short et al. is also being maintained.

Re 103 rejection of claim 3 over Henikoff ; Zaccolo et al.; Krokan et al. and Short et al. as applied to claim 2 above further in view of Lutz et al. and Cosstick & Vyle

4. Since rejection of claim 2 over Henikoff ; Zaccolo et al.; Krokan et al. and Short et al. is being maintained, hence the rejection of claim 3 over Henikoff ; Zaccolo et al.; Krokan et al. and Short et al. further in view of secondary references Lutz et al. and Cosstick & Vyle is also being maintained.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 1, 4-7, 12-13 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Henikoff (1990) Nucl. Acid Res vol. 18, no 10 pp 2961-2966 in view of Zaccolo et al. (1996) J. Mol. Biol. 255: 589-603 (cited by applicant in IDS).

Regarding claim 1, Henikoff teaches a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence) of n base-pairs (see page 2961 materials and methods where colE1-derived phagemids are taught as vectors. By teaching cloning of insert into different phagemids such as pKUN19, pUC19 or pVZ1-- double-stranded polynucleotide sequence (master sequence) of n base-pairs are taught)

having a (+)-strand and a complementary (-)-strand (the phagemids are double stranded circular DNA molecules thus they have a (+)-strand and a complementary (-)-strand)

comprising the steps

(i) creating a collection of single-stranded fragments of the (+)-strand of the master sequence wherein all members of the collection have the same 5'-terminus and have a deletion in the 3'-terminus such that the collection represents (+)-strands with a length of n-1, n-2, n-3, ...nucleotides (Using helper phage single stranded DNA is made from the double stranded phage. Materials and methods par. 1 on page 2961. Any one of the DNA strands of the phagemid

can be labeled + and the complementary strand would be -. To make it easier o illustrate the invention using the language recited by applicant, Examiner is referring to the single stranded circular DNA that is used as template shown in top of fig. 1 as – strand. By performing steps 1 to 3 outlined in fig. 1 one will end with different nested deletions of the + strand wherein all members of the collection have the same 5'-terminus and have a deletion in the 3'-terminus. By using appropriate concentration, incubation temperature and length of incubation in presence of Exo III before the reaction is stopped one will end up with nested deletions such that the collection represents (+)-strands with a length of n-1, n-2, n-3, ...nucleotides. This will be after step 3) namely gap with Exo III shown in Fig. 1. So now we have the complete circular (–) strand starting template DNA to which the various 3' deletions of the (+) strand generated by the Exo III digestion are hybridized. The 5' end of all the deletions is same as shown in Fig. 1.;

(iv) synthesizing a (-)-strand by using the (+)-strand produced in step (iii) as a template strand (see page 2966 2nd par. where Henikoff teaches use of appropriate helper phage Mike or M13K07 to produce the single stranded sequence of both the + and - strands. Thus by this teaching covering use of both the + and – strands as templates, Henikoff teaches synthesizing a (-)-strand by using the (+)-strand produced in step (iii) as a template strand.

Regarding claim 1, Henikoff does not teach

(ii) introducing at least one universal or degenerate nucleotide at the 3'-terminus of the (+)-strands produced in step (i); He teaches producing the deletion mutants for sequencing.

Regarding claim 1, Zaccolo et al. teaches

(ii) introducing at least one universal or degenerate nucleotide at the 3'-terminus of the (+)-strands produced in step (i); (See abstract page 589 where Zaccolo et al. teach using mixtures of triphosphate derivatives of nucleoside analogs dPTP and 8-oxodGTP as substrates along with mixture of normal dNTPs for elongation by Taq polymerase during PCR resulting in random mutagensis. Since the (-) strand serves as template in the instant case the region that was newly synthesized in presence of the above mixture would result in random mutagenesis of the (+) strand). By teaching use of dPTP and 8-oxodGTP, Zaccolo et al. teaches introducing at least one universal or degenerate nucleotide at the 3'-terminus of the (+)-strands produced in step (i).

(iii) elongating the (+)-strands produced in step (ii) to the full length of the master sequence using the (-)-strand or fragments thereof as a template strand for the elongation (Zaccolo et al. teach PCR (See page 591 last par where PCR is taught), and the template produced by method of Henikoff described above have the 3' deletions of (+) strand hybridized to the complete circular (-) strand template serves as primer for elongation. Thus in this situation PCR taught by Zaccolo et al. will meet the limitations of the above recited scenario) ;

(iv) synthesizing a (-)-strand by using the (+)-strand produced in step (iii) as a template strand thereby effecting mutations in the (-)-strand at the positions of the previous degenerate nucleotides compared to the master sequence (See whole article by Zaccolo et al. specially Abstract where incorporation of dP and 8-oxodG in (+) strand is taught to result in mutations at the positions in the (-)-

strand at the positions of the previous degenerate nucleotides compared to the master sequence.

Regarding claim 4, Zaccolo et al. teach wherein step (ii) comprises the elongation of the collection of single stranded fragments produced in step (i) with universal base or degenerate base by enzymatic or chemical methods (see page 591 last par. where Taq polymerase is taught as an enzyme that was used to incorporate degenerate base. Thus Zaccolo et al. teach elongation of the collection of single stranded fragments with degenerate base by enzymatic means.

Regarding claim 5, Zaccolo et al. teaches wherein terminal deoxynucleotidyl transferase or DNA polymerases or DNA/RNA ligases are used for elongation (Taq polymerase taught by Zaccolo et al. for elongation is a DNA polymerase).

Regarding claim 6, Zaccolo et al. teaches deoxyinosine as a nucleotide analog with promiscuous base pairing property is used as a universal nucleotide in step (ii) (See page 597 par. 2 where 2' –deoxyinosine and dITP are taught).

Regarding claim 7, Zaccolo et al. teaches wherein N4-hydroxy-2'-deoxycytidine (see page 597, par. 2), 8-oxodeoxy- guanosine triphosphate (8-oxo-G) (see abstract where 8-oxodGTP is taught) or a nucleotide analog with promiscuous base pairing property is used as degenerate nucleotide in step (ii).

Regarding claim 12, Zaccolo et al. teaches , wherein the elongation in step (iii) is effected by a PCR reaction (See page 591 last par. where PCR is taught).

Regarding claim 13, Zaccolo et al. teaches, wherein step (iii) comprises the synthesis of a (-)-single stranded plasmid polynucleotide sequence from a double-stranded plasmid harboring the master sequence using a primer which anneals downstream of the (+)-strand of the master sequence, and annealing of this (-)-ss-plasmid polynucleotide sequence with the (+)-strand produced in step (ii), and elongation of the (+)-strand (see page 600 Section Mutagenesis where Zaccolo et al. teaches use of appropriate sense and antisense primers for PCR. Thus by teaching appropriate primers Zaccolo et al. teaches a primer which anneals downstream of the (+)-strand of the master sequence, and annealing of this (-)-ss-plasmid polynucleotide sequence with the (+)-strand produced in step (ii), and elongation of the (+)-strand)

Regarding claim 15, Zaccolo et al. teaches, wherein a PCR amplification is used after step (iii) in order to synthesize a (-)-strand complementary to the (+)-strand produced in step (iii), thereby effecting a double-stranded master sequence carrying mutations (see details provided above for claim 1, step (iv).

By completing extension of Exo III generated templates produced after step 3 of Henikoff procedure in presence of the mixture of dNTPS taught by Zaccolo et al. with Taq polymerase. One would end up with a full length nicked circular species. Here the (-) strand is full circle while the (+) strand that contains the degenerate bases extended till it reaches the base adjacent to the 5' end will have the nick. The 5' P of the nick can be ligated to the 3'OH of the extended sequence by use of T4 DNA ligase taught by Henikoff et al. resulting in covalently closed double stranded hybrid where (-) strand is parental. The (+)

strand is mutant. The mutant (+) strand when serves as a template during the PCR reaction taught by Zaccolo et al. results in effecting a double-stranded master sequence carrying mutations (see page 591 par. 3 where in vitro mutagenesis of target DNA by PCR is taught. Also see page 600 section entitled mutagenesis provides details of how the two stage mutagenic PCR is conducted the first PCR in presence of mixture of dNTPS and degenerate dNTPS followed by second PCR where no degenerate dNTPS are used)

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to practice the method of Zaccolo et al. in the method of Henikoff . The motivation to do so is provided by both Henikoff and Zaccolo et al.

Henikoff et al teach use of Exo III enzyme to produce templates where the 3' deletions of the (+)single strand is hybridized to (-) strand circular DNA. These partially double stranded molecules have a free 3'OH group that can be elongated by polymerase such as TaqI.

Zaccolo et al. teach use of incorporation of degenerate nucleotides by TaqI in the elongating strand during PCR. During subsequent cycles of PCR this results in transition mutations. So one of ordinary skill immediately realizes that they can use the ExoIII generated molecules produced in the Henikoff procedure and use them in the PCR amplification taught by Zaccolo et al. to generate random mutants.

The advantage is obvious to one of ordinary skill because now they do not have to know the sequence of the master (+) strand or (-) strand and yet they are

able to generate random mutations in each location corresponding nucleotide of master (+) strand by a PCR mutagenesis protocol without synthesizing or hybridizing even a single primer, resulting in a method that can be applied universally to any unknown sequence that is cloned into the phagemids taught by Zaccolo et al. Since the method requires no artificial synthesis of primer, it results in significant savings in cost to the practitioner.

7. Claims 2, 8-11 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Henikoff and Zaccolo et al. as applied to claim 1 above further in view of Krokan et al. (US pat. 6,713,294 B1 with the national stage application 09/101,368 entry date of March 3, 1999 published first as WO97/25416 on July 17, 1997) and Short et al. (US pat. 6238884 B1 filed March 9, 1999).

Regarding claim 2, Henikoff and Zaccolo et al. teach method of claim 1, but do not teach wherein the collection of single-stranded fragments in step (i) is created by incorporating nucleotide analogs and subsequent cleavage in alkaline or acidic solution.

Regarding claim 2, Krokan et al. teaches incorporation of uracil--a nucleotide analog into DNA (see col. 20 line 65). They teach release of uracil by use of Uracil DNA glycosylase from ss or ds DNA containing uracil (see col. 4 line 10). They also teach use of DNA glycosylase to generate an abasic site and subsequent cleavage (at pyridines) by alkaline solution or AP endonuclease. (see col 1 lines 21-44 and col. 18, lines 30-31). Thus Krokan et al. teach incorporating nucleotide analogs and subsequent cleavage in alkaline or acidic solution.

Regarding claim 8, Zaccolo et al. teach use of oligonucleotides but they do not teach use of oligonuclotides of the type recited in claim 8.

Krokan et al. teach use of a uracil containing primer to obtain uracil-containing DNA (see col. 16 line 64-67). This uracil-containing DNA is taught to be cleaved by Uracil DNA glycosylase (see col. 16 lines 60-62). By teaching a uracil containing DNA primer, Krokan et al. teach an oligonucleotide of the general formula

$p(U)_a(N)_b*(S)_c$[TERM]

with

p = 5'-phosphate or hydroxy-group or any chemical group capable of forming diester bonds (since the primer is taught to prepare uracil containing DNA therefore the primer must necessarily contain p = 5'-phosphate or hydroxy-group or any chemical group capable of forming diester bonds during the polymerization process, otherwise the primer could not be use to form the DNA)

U = universal or degenerate bases

a = arbitrary integral number from 0 to 10000. In this example of oligo taught by Krokan et al. a=0

N = mixture of four bases (A/T/G/C (standard nucleotides)) and b = arbitrary integral number from 0 to 100 (since primer is taught generally length of primers is between 10-50 nucleotide long. Thus by teaching a primer the oligo taught by Krokan et al meets the limitation of b= 0-100.

* -- cleavable group such as phosphothioate bonds in phosphothioate nucleotides. In the oligo taught by Krokan et al, no phosphothioate nucleotides are present hence the cleavable groups are necessarily absent.

S = standard nucleotide or nucleotide analog and c = arbitrary integral number from 0 to 100. In the example taught by Krokan et al. Uracil meets the limitation that S = standard nucleotide or nucleotide analog and c = arbitrary integral number from 0 to 100.

Regarding claim 8, Krokan et al do not specify the location where the uracil is incorporate in the primer. Since b is some number between 10-50 in the above situation and a=0 therefore oligonucleotide taught by Krokan et al. meets the proviso that a+b>0,

Krokan et al. teach use of [TERM] = a dye terminator or any group preventing elongation of the oligonucleotide, (see col. 27 line 35 where use of Dye terminator is taught. Here the sequencing is done by dye terminator cycle sequencing. Thus teaching that the dye terminator taught by Krokan et al. is [TERM] = a dye terminator or any group that prevents elongation of the oligonucleotide and hence causes termination).

Hence Krokan et al. teach use of Oligo to introduce universal or degenerate bases (taught by Zaccolo et al.) to the collection of single- stranded fragments created in step (i).

Regarding claim 9, Short et al. teach site directed mutagenesis, cassette mutagenesis (see col. 43 lines 49 and 50) Thus by teaching the two methods, Short et al teach wherein the oligonucleotide is designed in a way that

(a) stop codons and/or

(b) amino acids which disrupt secondary structures,

are avoided in the collection of the mutagenized polynucleotide sequences.

Regarding claim 10, Zaccolo et al. teach wherein the oligonucleotide is designed in a way that

(a) transition mutations (Zaccolo et al. teach use of dPTP analog which yields transition mutations see abstract and several other base analog transition mutagens see page 590 par. 4)) or

(b) transversion mutations (Zaccolo et al. teach use of 8-oxodGTP which elicits transversions ---see page 591 par. 2),

are effected in the collection of the mutagenized polynucleotide sequences.

Thus by using the appropriate base analog taught by Zoccalo et al. as degenerate base in the oligo taught by Krokan et al. and Short et al. one would arrive at an oligonucleotide is designed in a way that (a) transition mutations or (b) transversion mutations are effected in the collection of the mutagenized polynucleotide sequences.

Regarding claim 11, Henikoff teaches wherein the single-stranded fragment created in step (i) which is not ligated with the oligonucleotide is removed using exonuclease (see above for claim 1 where Exo III is taught as an exonuclease that degrades DNA starting from 3' end).

When oligo that has 3' end blocked by terminator dye (taught by Krokan et al. is used) and it is ligated to the single stranded fragment created in step (i). Then all the molecules that have the oligo ligated to them will no longer have a

free 3'OH group that can be attacked by Exo III. Thus this species of molecules will be resistant to Exonuclease digestion, where as the single-stranded fragment created in step (i) which is not ligated with the oligonucleotide will still have a free 3'OH group hence it will be removed using exonuclease.

Thus by teaching Exo III, Henikoff teaches wherein the single-stranded fragment created in step (i) which is not ligated with the oligonucleotide is removed using exonuclease.

Regarding claim 14, Zaccolo et al.; teaches wherein step (iii) comprises the synthesis of a (-)-single-stranded plasmid harboring the master sequence. They also teach use of appropriate primers. Thus Zaccolo et al. teaches a primer which anneals downstream of the (+)-strand of the master sequence and Zoccalo teaches elongation in the presence of analogs and degenerate nucleotides but does not recite use of uracil

Regarding claim 14, Krokan et al. teaches elongation in presence of uracil and standard nucleotides (see col. 20 example 2 where uracil containing DNA labeled with tritium is taught. By teaching this uracil containing DNA. Krokan et al. teach the elongation was in presence of uracil and standard nucleotides)

and after the elongation of the (+)-strand produced in step (ii), the uracil carrying (-)- single-stranded plasmid is digested with uracil glycosylase (see claim 2 above where use of uracil glycosylase is taught by Krokan et al. Thus in the instant case the (-)single-stranded plasmid will contain the uracil and this will be digested by uracil glycosylase.

It would have been prima facie obvious to one of ordinary skill in the art to use the method of Krokan et al. in the method of Henikoff and Zaccolo et al. at the time the invention was made. The motivation to do so is provided by Zaccolo et al.; and Short et al.

Incorporation of degenerate nucleotides, (as taught by Zaccolo et al.), during elongation results in random mutagenesis over the entire region being elongated.

However if one was interested in mutating a specific enzymatic domain of an enzyme while ensuring that other parts of the enzyme were unchanged. Then if one were to use the oligos that had the degenerate nucleotides/etc predesigned in the oligonucleotide then one could make targeted mutants by designing the oligos targeted for specific parts of the sequence to be changed. This is corroborated by a patent issued to Short et al. that teaches directed evolution by performing site saturated mutagenesis where oligos are used to direct mutation of the desired amino acids. (See Short et al. section labeled Saturation Mutagenesis col. 34 lines 54-67 where use of proprietary codon primers (oligos) is taught to introduce point mutations).

Zaccolo et al. teach use of degenerate nucleotides that are potent mutagens with known mode of action. Thus by using appropriate degenerate nucleotides in the specific location of the oligonucleotide, one can design oligos that will generate transitions or transversions in those specific locations.

In view of the above teaching one of ordinary skill can design oligos that would be targeted to the specific part of the sequence to be mutated.

8. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Henikoff ; Zaccolo et al.; Krokan et al. and Short et al. as applied to claim 2 above further in view of Lutz et al. (2001) Nucl. Acids Res. Vol. 29 No 4 e 16; and further in view of Cosstick & Vyle (1990) Nucleic acids Res. Vol. 18 No 4 pp 829-835.

Regarding claim 3, Henikoff ; Zaccolo et al.; Krokan et al. and Short et al. teach method of claim 2, wherein the nucleotide analog is an alpha-phosphothioate nucleotide and oxidative cleavage is achieved by iodine at the phosphothioate bonds.

Regarding claim 3, Lutz et al. teach wherein the nucleotide analog is an alpha-phosphothioate nucleotide (page 3 of 7 where DNA spiking by PCR in presence of alpha-phosphothioate nucleotide is taught). Lutz et al. teach use of Exo III for cleavage of DNA. But they do not teach cleavage by iodine at the phosphothioate bonds.

Regarding claim 3, Cosstick & Vyle teach oxidative cleavage is achieved by iodine at the phosphothioate bonds (see abstract where they state the P-S bond is readily cleaved by solution of iodine).

It would have been prima facie obvious to one of ordinary skill in the art to practice the method of Lutz et al in the method of Henikoff ; Zaccolo et al.; Krokan et al. and Short et al. at the time the invention was made. The motivation to do so is provided by Cosstick & Vyle.

Cosstick & Vyle state "oligonucleotides containing phosphorothioate linkages have proved useful tools for the study of DNA processing enzymes and

DNA structure. In addition their increased resistance to nuclease activity suggests a potential application in the antisense approach to viral chemotherapy" (see page 829 par. 1 introduction). They go on to state "Procedures have been developed which enable ----to be incorporated into dinucleotide phosphate analogues using phosphoramidite chemistry. The synthetic methods are compatible with automated, solid phase synthesis of oligodeoxynucleotides -----. The ease with which the modified linkage is cleaved in the presence of silver ions or iodine solutions suggests that the incorporation if 3'-S-phosphorothioate linkages into oligonucleotide primers and their subsequent chemical cleavage may prove a useful technique for the 'nicking' and manipulation of DNA.

Conclusion

9. All claims 1-15 remain rejected over previously cited art.

10. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUCHIRA PANDE whose telephone number is (571)272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Kenneth R Horlick/
Primary Examiner, Art Unit 1637

Suchira Pande
Examiner
Art Unit 1637